Evaluation of culture methods for investigation of *Salmonella enterica* serovar ecology in feces.

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Summary: The present study evaluated culture methods to optimize detection and isolation of a wide range of *Salmonella* serovars. Fecal samples were obtained from cows, horses and pigs. Select samples were seeded with antibiotic resistance carrying *S.* Typhimurium and *S.* Choleraesuis as positive controls. Two pre-enrichment (BP, GN), 3 selective enrichment (RV, SC, T broths) and 5 selective plating (BGS, XLT4, HE, RA, CAS) media were evaluated. Colonies were counted on selective agars as presumptive *Salmonella* (PSC) or non-*Salmonella* (NSC). Differentiation of PSC and NSC was more difficult on BGS and HE and best on CAS. Both pre-enrichment media increased PSC detection. RV and SC inhibited NSC more than T. RA detected morphological differences among serovars. No single method appeared adequate to investigate *Salmonella* serovar ecology. Future work will use two selective enrichments, RV and SC, after BP pre-enrichment with isolation on XLT4 and CAS followed by RA for differentiation indices.

Keywords: selective media, isolation, ecology, serovar differentiation

Introduction: Differences among *Salmonella* serovars (over 2300 identified) exist for host specificity, invasiveness and virulence. However, research focused specifically on *Salmonella* serovar ecology in food animals and their environments in limited. Similar growth requirements for bacteria in the *Enterobacteriaceae* family make it difficult to use culture methods to distinguish *Salmonella* from other enterobacteria, much less differentiate among serovars (Arroyo and Arroyo, 1995). Enrichment and cultivation media, as well as combinations, affect sensitivity of recovery and serovars recovered (Arroyo and Arroyo, 1995; Dusch and Altwegg, 1995). The ability to isolate *Salmonella* serovars is key to determining their presence in animals and environments and to developing molecular methods to differentially track them. The present study evaluated culture methods to optimize detection and isolation of *Salmonella* serovars from feces.

Materials and Methods: Fecal samples were obtained from cows (n=3), horses (n=3) and pigs (n=6). All fecal samples were suspended in phosphate-buffered saline (pH 7.2, 1 in 10 w/w) and homogenized for 3 minutes prior to media inoculation. Two pre-enrichment (buffered peptone water [BP], GN broth, Hajna

[GNH]; both incubated 24 h, 42°C), 3 selective enrichment (Rappaport-Vassiliadis [RV] broth 24 h, 42°C; selenite cystine [SC] broth and tetrathionate [T] broth both 24 h, 37°C) and 5 selective plating (brilliant green sulfa [BGS], xylose lysine tergitol 4 [XLT4], Hektoen enteric [HE], Rambach [RA], Chromagar Salmonella [CAS]; all 24 h, 37°C) media were evaluated. *Salmonella enterica* serovars Typhimurium χ4264 (nalidixic acid resistant) (Ty) and Choleraesuis χ3246 (streptomycin resistant) (Ch) were inoculated individually and in combination to pre-enrichment media and seeded (at approximately 10^3-10^4 CFU/ml) in select fecal samples (n=4) as positive controls. Bacteria in all samples were quantified prior to pre-enrichment, after pre-enrichment and selective enrichment; 3 serial dilutions (1 in 10 v/v ½ strength peptone water) were plated on tryptone soya, MacConkey's and selective plating agars. Colonies were counted on selective agars as presumptive *Salmonella* (PSC) or non-*Salmonella* (NSC). Statistics (significance $P \leq 0.05$) were run separately on natural and seeded fecal samples by ANOVA. Pure culture samples were compared using paired *t*-tests.

Results: There were no differences in growth of Ty and Ch pure cultures, except for lower Ch PSC on XLT4. When Ty and Ch were grown in mixed culture (initial counts 4.30 and 4.72 log CFU/ml, respectively), Ty outgrew Ch in BP and SC. The pairing of pre-enrichment with selective enrichment media resulted in growth differences with higher PSC for Ty in BP-SC, BP-TT, GNH-RV, and GNH-SC; but higher Ch PSC in BP-RV. Natural and seeded fecal sample results are presented in following table. There was a tendency for NSC to be higher with GNH especially when followed by RV and TT. Lower NSC were counted on HE compared to other plating agars.

Results of presumptive detection of *Salmonella* in natural and seeded fecal samples.

	Significance of effects (P≤)[a]						
Samples	**P**	**S**	**A**	**P*S**	**P*A**	**S*A**	**P*S*A**
Natural fecal (n=12)							
PSC	NS	0.07	0.05	0.001	NS	NS	NS
NSC	NS	NS	0.07	0.001	NS	NS	NS
Total	NS	NS	0.03	0.001	NS	NS	NS
Seeded fecal (n=4)							
PSC	NS	NS	0.01	0.007	NS	NS	NS
NSC	NS	NS	NS	NS	NS	NS	0.05
Total	NS	NS	NS	0.001	NS	NS	NS

[a] Effects P = pre-enrichment, S = selective enrichment and A = agar; '*' indicates interaction.

Discussion: Current culture methods for the detection and isolation of *Salmonella* are time and labor intensive. One of the original objectives was to determine whether these methods could be shortened. Based on our results a single pre- or selective enrichment step may be enough to detect *Salmonella* by PCR, currently

being investigated. However, it does not appear that culture methods can be shortened for isolation or serovar ecology. PSC were easier to identify on CAS, as were H_2S producing colonies on XLT4. We previously observed different colony morphologies for *Salmonella* serovars on RA; pig samples had the greatest variety of PSC morphologies on RA, especially with SC. All methods favored growth of Ty over Ch in mixed culture, unlikely to be a concern for detection but a potential problem for investigating serovar ecology. Because XLT4 is the primary selective plating agar used, occurrence of Ch in environmental samples may be underestimated. Counts were 10 to 100 fold lower for Ch on XLT4 than on other selective plating media. No single culture method or combination was superior to any other. Future investigations of *Salmonella* serovar ecology in our lab will use BP, both RV and SC with CAS and XLT4 for primary isolation followed by RA as a diversity indices.

References:

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